NATIONAL ACADEMY OF SCIENCES

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The Safety of Polyoxyethylene Stearates for use as Intentional Additives in Foods

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Polyoxyethylene Stearates
for Use as
Intentional Additives in Foods

A Report by the Food Protection Committee
of the Food and Nutrition Board

July 1953

National Academy of Sciences—
National Research Council

publication 280

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Preface

In 1951, the Food Protection Committee formulated eleven basic principles for the evaluation of the safety of food additives. In the light of these principles it then commenced a review of the available evidence concerning the safety of emulsifying agents in processed foods. A detailed study of this particular group of additives was undertaken because of the controversy over some of the emulsifying agents resulting from the FDA Hearings to establish standards for bread and ice cream, and because of the need for testing the usefulness of these general principles when applied to specific problems.

In a statement of November 9, 1951, the Committee reported that the available data were insufficient to permit a final judgment as to the safety of certain emulsifiers. Subsequent study led to a report in December, 1952, on The Safety of Mono- and Diglycerides for Use as Intentional Additives in Foods. The present report summarizes evidence, available to date, concerning polyoxyethylene stearate-type emulsifiers together with an evaluation of the significance of these facts with respect to the question of safety. Although the Committee chooses, as a rule, to deal with type-chemical terminology rather than trade names, circumstances in the present case necessitate primary reference to the materials in question by trade names.

Contents

I. The Properties of Polyoxyethylene Stearates

Polyoxyethylene stearates are emulsifying agents manufactured and sold under various trade names for use in foods. There are two general methods of manufacture of this type of compound. In the method typified by "Repco" and "Sta-soft," the prefabricated polyethylene glycol is esterified with commercial stearic acid. "Myrj 45"*, on the other hand, is produced by the direct action of eight mols of ethylene dioxide with one mol of commercial stearic acid.

Myrj 45 is thus a partial ester of commercial stearic acid and mixed polyoxyethylene diols, and contains approximately 44 percent fatty acid and 53.2 percent oxyethylene. Upon saponification with alkali or enzymatic hydrolysis with lipase, the ester is converted to the original stearic acid and a mixture of polyoxyethylene diols.

Myrj 45 is a white, soft, waxy or pasty solid, melting in the range of 25-29°C; it is insoluble but dispersible in water, and in practice may be premixed with shortening or other food ingredients, or added separately.

The ethylene oxide reaction is repetitive, steps of the reaction being indicated by:

1) $R \cdot COOH + (CH_2)_2O \longrightarrow R \cdot COO \cdot CH_2 \cdot CH_2 \cdot OH$

2) $R \cdot COO \cdot (CH_2)_2OH + n(CH_2)_2O \longrightarrow R \cdot COO \cdot (CH_2 \cdot CH_2 \cdot O)_n \cdot CH_2 \cdot CH_2OH$

The repetitive nature of the polymeric addition of ethylene oxide will lead to the formation of esters of a mixture of polyoxyethylene diols of varying molecular weights whose distribution is predicted by Flory's application of the Poisson distribution to this type of reaction (9). The calculated weight percent of diols based on Myrj 45 is given in the following table, which also includes the determined weight percent of the molecularly distillable portions of this substance lending themselves to analytical fractionation:

* The content of Section I is based largely on information provided by the Atlas Powder Company. The Committee has considered evidence indicating that Sta-soft and Repco are chemically similar to Myrj 45 (36), but there is no way of identifying other than by trade name the material used in any of the feeding and toxicity studies reviewed here. Insofar as products similar to Myrj 45 bearing different trade names can be chemically identified with Myrj 45, the content of this report will apply also to them.

Distribution of Polymeric Glycols in the Ester Polyoxyethylene (8) Stearate — Myrj 45

n	Calculated Wt. % of Polyol Based on Myrj 45	Determined Wt. % of Polyol Based on Myrj 45
1. (ethylene glycol)	0.009	0.04 to 0.06
2. (diethylene glycol)	0.105	0.1
3. (triethylene glycol)	0.55	0.4 to 0.6
4. (tetraethylene glycol)	1.57	1.5
5.	3.37	2.7
6.	5.59	7.5
7.	7.55	5.7
8.	8.58	
9.	8.40	
10.	7.22	
11.	5.55	
12.	3.84	not
13.	2.42	distillable
14.	1.40	
15.	0.76	
16.	0.37	
17.	0.17	
18.	0.075	

Experimental data are tabulated above for polymers from n=1 to n=7. The maximum values for ethylene glycol are based on a non-specific test, i.e., chromotropic acid analysis. Isolation of a minute quantity of polyol yielding a crystalline dibenzoate derivative has confirmed the presence of ethylene glycol. The polyethylene glycols have been estimated from analytical constants (hydroxyl number, refractive index) of various fractions. Diethylene glycol and triethylene glycol were fractionated by means of a Todd column and the higher polyethylene glycols (up to hepta-) were roughly separated by molecular distillation.

Myrj 45 is prepared in the presence of an alkaline catalyst which favors ester interchange at the elevated temperature of synthesis. It is not a "monostearate," but rather an equilibrium mixture of mono- and di-esters and the corresponding free diols. The weight percents of the three types of constituents based on solvent fractionation of Myrj 45 are as follows:

Free polyol	16%-19% (19.3%)
Mono-ester	27%-35% (33.2%)
Di-ester	48%-55% (47.5%)

The figures in parentheses are calculated for equimolar quantities of polyol, mono- and di-ester. The above figures show that Myrj 45 contains approximately equimolar quantities of the three types of constituents.

No significant chemical change leading to the formation of new functional groupings is induced in Myrj 45 by the conditions of baking, as is demonstrated by the essential identity of infra-red spectra of untreated Myrj 45 and the product extracted from baked bread.

In regard to its surface activity, Myrj 45 in 0.1 percent concentration does not appear to alter the interfacial tension characteristics of bile or stomach juice mixtures simulating those appearing in the digestive processes (5). In this study, the interfacial tension of the system of 0.5 percent bile salt solution versus cottonseed oil containing 1.5 percent monoglycerides was not significantly affected by concentrations of Myrj 45 up to 0.5 percent. Also, within the physiological pH range of 5.0 to 8.0, and in the presence of bile salts, 1.5 percent Myrj 45 had no greater effect on surface tension than did corresponding concentrations of the monoglycerides.

II. The Possible Dietary Consumption of Polyoxyethylene Stearates

Variations in the diets of individuals make it impossible to predict accurately the quantity of polyoxyethylene stearates that may be consumed by any one person. It is possible, however, to estimate the average consumption per capita and the maximum forseeable daily consumption by an individual.

Polyoxyethylene stearates reach the consumer through processed foods in which they have been incorporated. The principal use of Myrj 45 as a food additive has been in bakery products such as breads, rolls, and doughnuts. The use of polyoxyethylene stearate-type emulsifiers in ice cream and other processed foods may be anticipated. When, however, the amount of polyoxyethylene stearate exceeds 0.34 percent in breads and rolls, 1.00 percent in sweet rolls and doughnuts, or 0.20 percent in frozen dairy products, their use is reported to become impractical. Actual use of the additives is probably less than the maximum percentages noted above.

The average daily per capita consumption (based on family food consumption surveys of 1948) for the above items may be represented as follows:

Bread and rolls	122 grams
Sweet rolls and doughnuts	15 grams
Ice cream	21 grams

On the assumption that each of these foods contains the maximum percentage for the practical use of the emulsifier, the average daily consumption of polyoxyethylene stearate as a continuing component of the diet is estimated to be in the order of 0.6 grams per person. The maximum potential dietary intake, based on 3,000 calories from bread containing 0.34 percent of the emulsifier, is estimated to be 3.7 grams.

Assuming that 600 grams of moisture-free food is consumed, these estimates would correspond to about 0.1 percent and 0.6 percent on a dry weight basis. On a body weight basis, the estimates would be approximately 9 milligrams per kilogram and 53 milligrams per kilogram. On a metabolic activity basis (weight $^{0.75}$), the estimates would be 25 milligrams per kilogram $^{0.75}$ and 150 milligrams per kilogram $^{0.75}$.

Inasmuch as the physiological results of feeding polyoxyethylene stearates appear primarily related to metabolism, and, like metabolism, the quantity of food consumed by different species follows a power function of body weight, dietary concentration appears to be a proper relationship for translation of results among species. For example, on a dry weight basis, a 2 percent diet for the rat compared with 0.1 percent for man would represent a factor of safety of 20 to 1. On a body weight basis, a 400 gram rat consuming 15 grams of a diet containing 2 percent polyoxyethylene stearate ingests 750 mg. per kg. which, compared to 9 mg. per kg. for man, would represent a factor of safety of 83 to 1. On a basis of weight $^{0.75}$, the rat would be getting 600 mg. per kg. $^{0.75}$ compared with 25 mg. per kg. $^{0.75}$ for man representing a factor of safety of 24 to 1. The dosage response comparison among species of test animals in relation to a reasonable factor of safety for humans is considered in this report to be fairly represented on a dietary percentage basis.

III. The Toxicology of Polyoxyethylene Stearates

Mortality

No effect on mortality rate was observed when rats where fed for two years on diets of ground commercial rat biscuits containing Myrj 45 added at levels of 2, 5, 10, and 25 percent (10). The results of shorter tests on rats (2, 13, 14, 24, 25, 26, 28, 34), rabbits (18, 34), mice (19), cats (27), chicks (46), humans (38), and dogs (1, 10) were also negative with respect to mortality.

Hamster feeding tests have been inconclusive. On a Fox-Chow basal diet for 106 days (2) or on a diet of natural ingredients (34), no mortality differences were observed when 1, 5, and 10 percent Myrj 45 were added to the ration. On bread diets, the mortality (32) was 50 percent and 100 percent on diets containing 5 and 15 percent glycerol monostearate; 52 and 56 percent on 5 and 15 percent Myrj 45; 30 percent on 15 percent lard.

Other investigators (35) observed similar mortality rates with bread diets containing Myrj 45 and Sta-soft. Whether the mortality obtained with hamsters on a bread diet was the result of the dietary supplement or the basal diet itself is obscure.

Inconclusive mortality data were also obtained with mice (3). Negative effects at 5 and 10 percent dietary levels of Myrj 45 cast doubt on the significance of 6/11 mortality at 2.5 percent, and 4/11 mortality at 15 percent Myrj 45 fed for 337 days. Factors unrelated to the diet may have been operative. However, a relatively flat dose-response curve and the small number of animals per group could account for these apparent irregularities.

Growth

When Myrj 45 was added to a hamster diet of natural ingredients at a 5 percent level for two weeks, followed by a 10 percent level for two weeks, no ill effects were observed (34). Similarly, in another hamster experiment (2), 1 to 10 percent Myrj 45 added to a Fox-Chow diet for 106 days had no adverse effect on growth pattern. However, when Myrj 45 was added to a bread diet at 5, 10, and 15 percent levels in a 39-week feeding test, the growth of weanling hamsters was apparently moderately depressed. In a short two-week feeding test with 10 and 25 percent Sta-soft added to a bread diet, growth was again apparently inhibited according to *ad libitum* feeding tests. Other investigators, using purified diets and *ad libitum* feeding methods, found growth inhibited by diets containing 5 and 15 percent of Myrj 45 and Sta-soft (37). A third study (32), using hamsters and *ad libitum* feeding, demonstrated growth inhibition with bread diets containing 5 and 15 percent Myrj 45, as well as 5 and 15 percent glycerol monostearate. In the latter investigation, the animals suffered anorexia "because of the taste of the breads" and also diarrhea which resulted in nutritional deficiency, dehydration, lowered resistance to infection, and terminal respiratory infection.

Growth data obtained with other species were either inconclusive or negative. Mice, fed *ad libitum* 2.5 to 15 percent of Myrj 45 in a diet for 337 days, gained less weight than control animals, which however were declared to be abnormally obese (3). In a two-year study on young rats, with dietary concentrations of 2 to 25 percent Myrj 45

added to a diet of commercial rat biscuits and fed *ad libitum*, growth was depressed in the males at a 25 percent level of the additive. The females failed to demonstrate this effect at the 25 percent level; in fact, the females at 10 percent showed significantly greater weight gains. In another *ad libitum* feeding experiment on a synthetic diet containing 25 percent Myrj 45 for 59 and 70 days (13), weight gain was less than that obtained by substituting hydrogenated oils (Crisco). In another investigation designed to repeat the latter observation (14), it was found that additional vitamin supplements eliminated the apparent depressive effect of the emulsifier on weight gain. This observation might indicate that the depressive effect was caused by diminished appetite as a result of a destructive action (31) of the emulsifier on one or more of the vitamins rather than by any toxic action of the Myrj 45.

In another study on rats (34), a normal growth pattern was observed when 5 to 20 percent of Myrj 45 was fed for 12 to 24 weeks. A two-year test (26) at 4 percent and a 499-day test (28) at 6 percent, similarly had no effect. The results of less severe tests in young rats (14, 24, 25), failed to show a deleterious effect of Myrj 45 on the growth pattern. Notable among these tests was one (25) which was carried on for three generations on a 4 percent Myrj 45 dietary level. In this study, no influence of the Myrj 45 diet on growth, fertility, or number or weight of offspring was observed.

Chick feeding tests, using 0.1 to 2 percent Myrj 45 for 7 weeks, were negative (46). Tests with dogs (1, 10), fed 5 and 10 percent of Myrj 45 for 19 months and 20 weeks, were also negative. Young cats fed 20 percent Myrj 45 for 6 months (27); young rabbits fed 4 percent for 4 months (18), and 10 percent for 4 weeks (34); and 7- to 24-week-old humans fed 0.7 grams per day in their formulas for 20 to 32 weeks (16) all exhibited normal weight gains.

The only reported studies of the effect of the Myrj 45 polyol in the diet are those in which rats were fed 6.4 percent and 12.8 percent of this substance (equivalent to 10 and 20 percent of Myrj 45) for six weeks (34), and 6 percent for 491 days (26). Animals in these tests gained weight normally.

In male rats receiving diets containing 30 percent lard (31), growth was markedly depressed when 15 percent Myrj 45 was substituted for an equivalent weight of sucrose in the diet. Females were not so affected, nor was the effect observed when 45 percent lard was fed as the control diet.

In the course of studies relating to the feeding of Myrj 45 under a variety of dietary conditions (31), it was observed that 2 to 15 percent Myrj 45 added to a diet containing 80 percent of the thiamine requirement inhibited growth and hastened the onset of polyneuritis. The tests indicated that the addition of Myrj 45 to the ration caused destruction of thiamine. Storage tests on rations with and without Myrj 45 fully confirmed this. In a period of a week, there was a reduction of only a few percent in the thiamine content of the basal ration and a 70 percent reduction with the addition of Myrj 45.

Although the weight of the evidence seems to indicate that polyoxyethylene stearate does not affect growth directly, the data are difficult to interpret because of insufficient notation of effects on palatability of the ration, on appetite of the animal, on species differences in response to basal diet, and particularly on nutrient balance (including energy). In no case was the metabolizable energy of either the control or test diets directly determined, although attempts were made to estimate the

caloric utilization by conventional calculations.

Organ Weights

Rats fed for two years on commercial rat-biscuit diets containing 2 to 25 percent Myrj 45 exhibited no deviation from the controls in liver, kidney, and testis weights (10). A 499-day test with 6 percent Myrj 45 and 6 percent Myrj 45 polyol (28) also revealed no changes in the weights of liver, kidney, testis, and adrenal. Negative findings are also reported for liver and kidney in the shorter-term experiments with lower dietary levels of Myrj 45 (24), in the three-generation test on rats (25), and for the liver, kidney, brain, heart, lung, and spleen of rats on 25 percent Myrj 45 for 59 days (13). Kidney weights were not significantly affected in the rat by 25 percent Sta-soft fed for 21 weeks (8). In the hamster, however, moderate increases were observed in the liver and kidney weights after a 10-week feeding period on a purified diet containing 5 and 10 percent of Myrj 45 and Sta-soft (37), while the spleen (37) and the testes (35, 37) were not affected. In a 106-day test on hamsters (2) fed 10 percent Myrj in a Fox-Chow diet, a significant decrease in liver weight was seen; kidney, heart, and spleen weights were not affected. Hamsters fed for 18 weeks on bread diets containing 5 and 15 percent Myrj (32) showed no weight changes in liver, kidney, spleen, testis, and ovary.

Acute and Short-term Toxicity

Rough estimates of the LD_{50} values were made (8) for single oral doses of Myrj 45 and Sta-soft in the hamster and rat, and for single oral (8) and intraperitoneal (21) doses in the rat. The results indicate a very low order of acute toxicity of these materials under these conditions. Due to a limitation in the volume of material that can be placed in a rat stomach, it was not possible to administer doses of Myrj 45 large enough to kill rats by its toxic action (8). There was some evidence that Sta-soft exhibits a moderately greater toxicity in the rat and the hamster than does Myrj 45, a difference suggested by, but not consistently reflected in, the feeding tests already described. In accord with the results of feeding tests, however, are the comparative susceptibilities of the hamster and rat to single oral doses. The LD_{50} for the rat is at least 2.5 times that for the hamster for both Myrj 45 and Sta-soft. Rabbit studies indicate only that the single oral lethal doses of both agents are greater than 12 ml. per kg.

The single oral LD_{50} of the polyol portion of the Myrj 45 in the rat was 25 ml. per kg. (8). Since this is less than half the LD_{50} ($>$ 68 ml. per kg.) of Myrj 45 itself under the same conditions and since the polyol fraction constitutes a little more than half (55.5 percent) of the Myrj 45 molecule, the acute toxicity of the latter appears to be due to its polyol component. A comparison of the results of feeding tests of Myrj 45 and its polyol throws no direct light on this point, since no mortality data were obtained in such tests (28, 34). It was observed, however, that 12.8 percent polyol in the diet of rats caused a diarrhea, whereas 20 percent Myrj 45 (molar equivalent of 12.8 percent polyol) had no such effect.

The single intraperitoneal LD_{50} of Myrj 45 polyol in the rat is reported to be 13 ml. per kg. (21), or half the corresponding value for oral administration (8). In these studies, the only statements regarding symptoms and death times were in connection with the intraperitoneal polyol-toxicity test (21). Here it was merely stated that "fatalities at the end of a 24-hour observation period were noted," that there was transient irritation at the site of in-

jection, and that the animals appeared depressed by large doses.

When Myrj 45 and Sta-soft were administered orally to hamsters in various single doses daily for eight days (10), the estimated LD_{50} values in terms of ml. per kg. per day were 16.9 and 16.2 respectively. Since none of the animals dying from Sta-soft and only two of those dying from Myrj 45 required all eight doses to kill them, it cannot be stated whether or not some of those which died might have done so following fewer doses than were actually administered. Body weights of the surviving animals, however, though initially depressed for about seven days after the last dose, subsequently did not differ significantly from the weights of the control animals. Since the LD_{50} values given for this manner of administration are of similar order of magnitude to those obtained for single oral doses, it may be concluded that there is little cumulative acute toxic action involved.

Two children, aged 11 months and 6 years, exhibited no adverse symptoms when given 4 grams of Myrj 45 per day for 14 and 16 days, respectively, as reflected by peripheral blood counts, urinalysis, fecal fat and nitrogen excretion, gastrointestinal radiographic series, and duodenal enzyme analyses (38).

Gross and Micropathologic Observations

Possibly deleterious effects were observed in rats fed Myrj 45 for two years (10). In this study, detailed histologic examinations were made of lung, heart, liver, spleen, pancreas, stomach, three levels of small intestine, colon, kidney, adrenal, gonads (and uterus), bladder, prostate, thyroid, parathyroid, leg bone and muscle, and bone marrow. The possibly significant effects of Myrj 45 in the diet, tabulated below, were limited to the bladder, stomach, and liver.

Myrj % of diet	Rats Started	Rats Survived	Rec'd in Path.	Microscopic Section	Bladder Concretions	Bladder Tumors	Liver cell Vacuol	Mild Hepatitis	Stomach Epithelial Hyperplasia
25	24	9	21	21	3	2	11	3	3
10	24	11	23	21	0	0	8	2	3
5	24	9	18	17	1	0	6	3	1
2	24	10	21	20	0	0	4	2	1
0	24	12	17	17	0	0	3	0	0

Gross changes probably attributable to Myrj 45 occurred in the bladder. Four concretions and two tumors were found in 83 Myrj 45-fed rats as compared with none in 17 control animals. Two stones that were analyzed proved to be calcium oxalate. Though the incidence of these findings among all animals fed Myrj 45 at levels of 2, 5, 10, and 25 percent is low, it becomes important when only the group of 21 rats on 25 percent Myrj 45 is considered, since both tumors and three of the concretions were found in this group. The fourth concretion occurred in the group receiving 5 percent Myrj 45. Thus the incidence of such bladder changes may be attributed to 25 percent Myrj 45 in the diet.

Possibly significant changes due to Myrj 45 also occurred in the stomach and liver (10). A slight hyperplasia of squamous epithelium was seen in eight of the Myrj 45-fed animals, and small erosions of the glandular mucosa were seen in four; these changes did not occur in any of the controls. A mild hepatitis was manifested in 10 of the Myrj 45-fed rats and in none of the controls. An increase in liver vacuolation that correlated with Myrj content of the

diet may be attributed to the fat in the diet, as shown by the 21-week feeding tests in which rats on 25 percent Sta-soft, lard, and mono- and diglycerides showed 100 percent incidence of "large-droplet fatty change" in the liver.

In a 39-week hamster-feeding study (8), bladder stones appeared in five of 41 hamsters on bread diets containing 5 to 15 percent of polyoxyethylene stearate (1/15 at 5 percent, 2/17 at 10 percent, 2/9 at 15 percent). The single stone that was analyzed was identified as calcium phosphate. No bladder stones were reported in a 10-week test on 132 hamsters on 5 to 15 percent Myrj 45 or Sta-soft (37), in 36 hamsters on 1 to 10 percent Myrj 45 for 106 days (2), nor in 46 hamsters on 5 and 15 percent Myrj 45 for 18 weeks (32).

Gross pathological changes seen in hamsters fed synthetic or bread diets containing 5 and 15 percent Myrj 45 or Sta-soft (8, 37, 47), in comparison with those fed lard or lard mono- and diglycerides, involved the gastrointestinal and urinary tracts and the testes. A distended intestinal tract, enlarged cecum, diarrhea, and inflammation of the base of the tail were typical findings, and were, in general, more severe at the higher dietary concentrations of the emulsifiers. The diarrhea and inflammation frequently developed within seven to ten days after the start of the experimental diet, but tended to subside in surviving animals after the fourth week (37). In 11 of 41 animals, a distortion of kidney contour was reported as compared to no distortion in control animals (8). Two groups of investigators observed a decrease in size of the testes (8, 37).

Extensive histopathologic studies were made on hamsters receiving 5 and 15 percent of Myrj 45, Sta-soft, and lard in a basal purified diet (47). In the intestinal tract, necrotic erosions of the mucosa in duodenum and ileum and an accumulation of hemosiderin in the cecum were seen. The liver also exhibited hemosiderosis, small hemorrhagic necrotic lesions, and mild sinusoidal congestion. In the kidney there was a collection of amorphous material in the tubules and some congestion and disintegration of renal capsules. As might be expected from the pathologic changes in the intestinal tract and kidney, blood was found in the feces and urine of occasional animals on experimental diets. The spleen was hemosiderotic and the testes exhibited a depression in spermatogenic activity. Significantly less damage, both in frequency and extent, was observed for the animals fed lard. Though these findings, as reported, tend to incriminate Myrj 45 and Sta-soft as the causative agents of some deleterious effects in hamsters, the following observations of the investigators (47) tend to lessen their importance: (a) no appreciable increase in pathologic changes was seen after the second week in animals fed up to 10 weeks on the experimental diets; (b) there was evidence that some animals became acclimated to the diet; (c) there was little or no correlation between extent or incidence of damage and the concentration of the emulsifier. As further evidence that the basal diet may be of importance, no gross changes were observed after 106 days of feeding hamsters on a Fox-Chow diet containing 10 percent of Myrj 45 (2). The liver, kidney, testis, stomach, duodenum, jejunum, ileum, cecum, and colon were examined histologically with no significant pathologic results being observed.

No evidence of gross or micropathologic changes attributable to polyoxyethylene stearates has been reported for species other than rats and hamsters. Mice and rabbits fed 4 percent of Myrj 45 in the diet for six weeks and four months, respectively (18, 19), and mice fed 2.5 percent Myrj 45 for 337 days (3), showed no gross

or microscopic changes in kidney or liver. In two studies on dogs, no gross pathologic changes attributable to Myrj 45 were observed (1, 10).

Blood and Urine Studies

Various investigations have been made on the effects of feeding Myrj 45 on the cellular and non-cellular components of the blood and urine of animals. In none of the studies was there any significant difference between the Myrj 45-fed animals and the controls with respect to blood constituents, including red and white cells in the mouse (19), red and white cells, urea nitrogen, blood sugar, cholesterol; hemoglobin, non-protein nitrogen, and serum sodium and potassium in the rat (10, 24, 25, 26, 34), red and white cells, hemoglobin, blood sugar, urea nitrogen, non-protein nitrogen in the rabbit (18, 33), red blood cells in the hamster (32), red and white cells, hemoglobin, non-protein nitrogen, urea nitrogen, and creatinine in the dog (1), red and white cells, hemoglobin, albumen, globulin, calcium, phosphorous, non-protein nitrogen, Vitamin A, and carotene in the human (6, 28). The most convincing of these negative results may be seen in the three-generation-plus-16-months test of rats on 4 percent Myrj 45 (25), the six-month test on rats at 20 percent (34), the eight-week test on rats at 25 percent (10), and the 20-week test on dogs at 10 percent (37).

Myrj 45, like other fatty acid esters, produced a clear, acid urine in rabbits; the polyol fraction alone had no effect (8, 34). No abnormal effects on the urine were observed in hamsters (32), children (38), or adult humans(6).

Gastrointestinal Function

Rats fed 25 percent Sta-soft for 21 weeks (35) and 20 percent Myrj 45 for six months did not show any diarrhea (34), nor did rats fed 25 percent Myrj 45 for eight weeks (10, 14). These high concentrations of Myrj 45 and Sta-soft generally caused the rats to excrete light gray-brown to white feces which, though well-formed, were somewhat less firm and more bulky than normal (8, 14). The water content of the feces of rats fed 25 percent Myrj was 21 percent above the controls after four weeks but subsided to 13 percent above after eight weeks (10). Rats fed 12.8 percent of the Myrj 45 polyol (equivalent to 20 percent Myrj 45) developed diarrhea within several days, though no diarrhea was caused by 6.4 percent (34).

No change in character of stool was noted when two dogs were fed 10 percent Myrj 45 for 20 weeks (1), nor when two children were given 4 grams of Myrj 45 per day for two weeks (38). No influence on gastrointestinal motility was observed in adults given 3.5 grams of Myrj 45 with a barium sulfate test meal (29) or in the children given 4 grams per day (38).

Addition of 5 and 10 percent of Myrj 45 to a diet of natural ingredients (34); or 1 to 10 percent of Myrj 45 to a Fox-Chow diet (2) did not cause diarrhea in the hamster. Addition of 5, 10, and 15 percent Myrj 45 or Sta-soft to purified diets (37) or bread diets (8, 32) caused diarrhea. However, monoglycerides also exhibited this effect in a bread diet with this species.

No effect on hydrochloric acid, total acidity or mucus in the gastric contents was observed in the 12 human adults receiving 3.5 grams of Myrj 45 in a test meal (29). The two children (38) receiving 4 grams of Myrj 45 per day for two weeks exhibited normal pancreatic enzyme activity (amylase, lipase, and trypsin). Six grams of Myrj 45, added to a therapeutic pancreatic powder supplement in the diet of a patient with pancreatic deficiency, did not affect the activity of the powder (6).

High-fat diets, in general, tend to increase

moderately the bacterial content of feces, although such changes are not considered of pathologic importance. Myrj 45, at a level of 25 percent in a rat diet, increased the bacterial count of rat feces by 30 percent. In this respect it was not significantly different from a 25 percent Crisco diet (10). Myrj 45 added at a 5 percent level to a hepatonecrogenic diet had no effect on the bacterial content of intestinal content or feces (12).

Evidence fails to indicate that the absorption of other fats in the diet is not influenced by the incorporation of Myrj 45. The total fat excretion in relation to total fat ingestion by rats receiving 25 percent of Myrj 45 was compared with that by control rats and by those fed 25 percent of other fats and emulsifiers. It was found that 24 percent of the total fat was excreted by rats consuming the Myrj 45, while 14 percent was excreted by rats ingesting Crisco or the control diet. High fat excretion was observed in all stearic and palmitic acid compounds (mono- and tristearin, mono- and tripalmitin) (10). In the case of Myrj 45, this finding is supported by the observation that 40 percent of its stearate component is not absorbed by rats (14). Further confirmation is found in an experiment in which olive oil was administered orally to rats. The addition of 6 percent Myrj 45 had no effect on absorption of the fat from the intestines (44). The administration of six grams of Myrj 45 to two human adults for three and eighteen days, respectively, following pancreatectomy, did not alter the fecal fat excretion pattern (6). These findings do not support the hypothesis that these emulsifying agents should promote fat absorption by virtue of their surface activity, and are in accord with the finding that Myrj 45, in concentrations up to 0.1 percent, did not alter the surface tension of systems containing cottonseed oil and bile, or of gastric juice taken from human subjects.

Metabolism of Polyoxyethylene Stearates

The absorption of the stearate fraction of both Myrj 45 and glycerol monostearate was about 60 percent when each was fed at a 25 percent level in the diet of rats (14). When 1.67 percent of Sta-soft was incorporated in the diet of rats, 49 percent was absorbed, and 24 percent was estimated to be metabolized (33).

In other studies, analyses for the polyoxyethylene fraction were made of the feces and urine of infant males receiving 0.7 gram per day of Myrj 45 (16). In 5 to 10 tests on each of four infants, the average urine content of the polyol was 54.4 to 63.8 percent of the total intake, while the fecal content ranged from 31.5 to 38.4 percent. The total amount accounted for averaged 92.3 to 100.5 percent of the ingested polyol. The results with four adults ingesting 54 to 108 grams of Myrj 45 over a 12- to 18-day period were of the same order of magnitude (6). The average excretion in the urine was 66.5 percent and in the feces was 29.8 percent.

Whether the polyol of Myrj 45 or the intact emulsifier was administered, urinary excretion was essentially the same in tests on two adult male subjects. Approximately three-fourths of the excreted polyol appeared in the urine within 4.5 to 8 hours after the ingestion of the emulsifier. The excretion was complete in 24 to 32 hours (40, 41). Analysis of the polyoxyethylene moiety isolated from the urine showed that about one-fourth of the polyoxyethylene diols had been oxidized to hydroxy-acids without splitting the oxyethylene chain.

A preliminary study of the distribution of polyoxyethylene in tissues was made in three rats at the end of a 21-week feeding period on 25 percent Sta-soft (35). The blood contained 6 to 23 mg. per ml.; none

was found in the fat deposits and only traces in the carcasses, probably due to blood remaining in the tissues. The rapid excretion and high recovery observed in humans suggests that there is no significant storage in the body (6, 16, 40).

Metabolic studies on polyethylene glycol, the polyoxyethylene moiety of Sta-soft, show that it behaves similarly to the polyethylene fraction of Myrj 45. An average of 77 percent of a one-gram intravenous dose of the polyethylene glycol was excreted within 12 hours by three human subjects (39). When dogs were injected with 2 ml. per kg. intramuscularly or subcutaneously, 95 percent was recovered in the urine within 48 hours. In three human subjects receiving 5- to 10-gram oral doses of polyethylene glycol 400, an average of 44 percent was excreted in the urine in 24 hours. This result is in close agreement with that obtained with the polyol of Myrj 45 (41).

No ethylene glycol could be detected in the urine of human subjects receiving polyethylene glycol 400 intravenously or orally (39). The respiratory quotients of fasting rats were not affected by oral administration of Myrj 45 polyol. This result indicates, providing the material was absorbed, that the polyol is not oxidized in the body of the rat (17). When Myrj 45 polyol was fed to rats, it did not act as a precursor for liver glycogen (20).

In order to ascertain whether the ingestion of Myrj 45 might be responsible for the appearance of oxalic acid in the urine, five human subjects were administered 4.5 grams of Myrj 45 per day for 10 to 12 days, and the urine was examined for oxalate (6). In contrast to the greater than 100 percent increase in one subject fed a high oxalate diet containing spinach, gelatin, and chocolate, there was no increase in urinary oxalate in these subjects. In a similar study on Sta-soft, no increase in urinary oxalic acid output was observed in five human subjects given 6.1 grams of Sta-soft daily for approximately one month (33).

Nutritional Efficiency

Caloric efficiency. The caloric contribution of polyoxyethylene stearates depends entirely on the absorbed stearate fraction of the molecule, since the polyoxyethylene moiety has been shown to be practically inert from a metabolic standpoint.

In an eight-week feeding test on groups of five rats (10), a group on 25 percent Myrj 45 consumed a 29 percent greater weight of food for each 100-gram gain in weight than did the control group. However, the weight gain of the Myrj 45-fed rats was only 78 percent of that of the controls. In accord with the greater food intake necessary to produce a 100-gram weight gain in the Myrj 45-fed rats is the finding that the weight gain per 100 calories of the Myrj 45 diet consumed was 70 percent of that for the control diet. This can probably be accounted for by the fact that about 40 percent of the stearate component of Myrj 45 is not absorbed.

The sluggish weight gain of hamsters fed Myrj 45 and Sta-soft has been attributed to a decrease in food efficiency, since the weight of experimental and control diets consumed per day was approximately the same (37). However, when the caloric contents of the diets were recalculated, and the 60 percent utilization of the stearate component is considered (14), the growth rate correlates well with caloric absorption.

In a study (14) in which the theoretical caloric contribution of the emulsifier was accounted for, the weight gain per 100 calories consumed for a 56-day period was 5.91 grams in rats on 25 percent Myrj 45 as compared to 5.46 grams on 25 percent hydrogenated vegetable shortening and 5.12 grams on 25 percent glycerol monostearate, all other dietary constituents being the

same. In this study, growth rates and food consumption varied between groups, and two assumptions were made: (1) The glycerol of the glycerides was included in caloric content estimation, but the polyol of Myrj was excluded. (2) Fecal fatty acid excretion was measured during one week, and the proportionate amount was subtracted in calculation of caloric ingestion for each diet. In view of the great individual variations encountered in these tests, and the deviations possible from the latter assumption, the above values may be considered to be in fairly close agreement.

Protein efficiency. The effect of dietary Myrj 45 concentrations up to 15 percent on protein efficiency in mice (3) and in dogs (1) was studied by relating weight increase to total nitrogen intake. The results of a 21-day test on groups of 10 male and female mice indicated no effect on this ratio in male mice on 10 percent, but a 29 percent increase in females on 15 percent Myrj 45. In a 14-week feeding test on weanling dogs fed a diet containing Myrj 45, the protein efficiency ratio for two experimental animals averaged 10.4 as compared to a 9.3 average for controls. The effect in both cases was likely due to the increased fat content of the experimental diet. This result well illustrates the weakness of weight-gain methods in measuring protein efficiency.

Absorption of other nutrients. In infants fed 0.7 gram per day of Myrj 45 in their formula, there was no interference with absorption and retention of nitrogen, calcium, phosphorous, or carotene (16). Replacement with Sta-soft of 3 percent of the fat of a Vitamin A-deficient diet fed to rats had no effect on the rate of development of deficiency symptoms, nor did the Sta-soft affect the absorption of a subsequent Vitamin A supplement (33).

Miscellaneous Observations

Allergenic reaction. Patch tests on a large number of human beings are necessary to assay the allergenic potential of an agent. However, a limited test on two guinea pigs (23) was negative in this respect.

Hemolytic action of the polyol. No studies have been reported on the possible direct hemolytic action of the polyoxyethylene moiety *in vivo* on high dietary levels of the emulsifiers. However, no hemolysis occurred when 0.1 ml. of defibrinated human blood was added to 10 ml. of physiological saline solution containing concentrations of Myrj 45 up to 10 mg. per ml. and allowed to stand for 18 hours at 37°C. (23).

Blood pressure. Two anesthetized dogs, injected intravenously with 5 ml. of a 5 percent Myrj 45 suspension, exhibited marked, but temporary, depressor response in blood pressure (23). In the same dose, the Myrj 45 polyol elicited no change in blood pressure of one dog.

IV. Evaluation

Extensive studies of the effects of Myrj 45 and some studies on closely related products have been made on various species of animals. These studies, diverse in nature, were determined by the special properties of the particular agent under scrutiny or by other circumstances. Many of the studies were conflicting; others did not contribute directly to the evaluation of safety. None of the toxicologic studies were accompanied by analytical results indicating the constancy of composition of the preparations used. The possibility that conflicting results of animal studies reflect variations in composition of the test substances should not be neglected.

Experimental findings given special consideration in the evaluation of the hazard of polyoxyethylene stearates were (a) changes in the urinary tract of rats (10) and hamsters (8, 47); (b) evidence of mild liver change in rats (10) and hamsters, including some hemosiderosis in the hamsters (47); (c) slight hyperplasia of the squamous epithelium of the stomach of the rat (10); (d) growth retardation and more rapid onset of peripheral neuritis in rats fed a diet low in thiamine content (31).

Studies on the metabolism of polyoxyethylene stearates and their effects on the blood, on diet efficiencies, on growth with complete basal diets, and on other physiological functions failed to reveal any further evidence against the safety of the additive.

The most disturbing observation in animals consuming diets containing polyoxyethylene stearates was the incidence of bladder stones in one study on hamsters (8) and in one on rats (10), and the occurrence of similar findings in rats fed other polyoxyethylene emulsifiers (8, 13). In assessing the importance of this finding with respect to the safety of polyoxyethylene stearates, the following information is pertinent:

1. Though the incidence of bladder stones in rats and hamsters on polyoxyethylene stearate diets was quite low, there appeared to be a definite association between this finding and the presence of the emulsifier in the diet in two studies. Four bladder stones were found in 96 rats (10) and five in 41 hamsters (8) fed polyoxyethylene stearates, while none were found in animals serving as controls in the feeding tests. The single stone that was analyzed in the hamster study was identified as calcium phosphate. Two bladder stones that were analyzed in the rat study proved to be calcium oxalate.
2. Two of the bladder stones appearing in rats fed 25 percent Myrj 45 were associated with bladder tumors, but the limited extent of such an occurrence does not justify a conclusion that the two effects are related.
3. Bladder stones have been reported in rats fed other polyoxyethylene-type emulsifiers, but not in animals fed emulsifiers lacking the polyoxyethylene moiety (8, 13).
4. Other investigators, using mice, dogs, rats, and hamsters, reported no bladder stones after long-term feeding tests with polyoxyethylene stearates.
5. No clinical indication of injury in the urinary tract was observed in men ingesting 4.5 grams of polyoxyethylene stearates per day for as long as 17 months, or 6

grams per day for 10 months. Urinary oxalate was not found to be increased in these subjects.

6. Bladder stones have also been reported in rats receiving ethylene glycol and diethylene glycol for a period of two years (30). Theoretical considerations and actual chemical analyses indicate that Myrj 45 contains 0.01 to 0.06 percent ethylene glycol, and 0.1 percent diethylene glycol. The average concentration in the human diet, based on 0.1 percent of the emulsifier, is estimated at 0.00001 to 0.00006 percent ethylene glycol and 0.0001 percent diethylene glycol. According to rat experiments, these concentrations are still below those which have been shown to produce bladder stones. If the rat and hamster could partially metabolize polyoxyethylene stearates to the lower glycols, an explanation for the development of stones in these species might be available. If not, it will have to be assumed that there is a relationship between the polyol itself and the appearance of stones.

In order to establish the maximum dietary level in which there is no causal relationship between the level of polyoxyethylene stearate and disorders of the urinary tract, further investigations are necessary in which sufficient numbers of animals would have to be used to permit statistical analyses of the data. In the investigations to date, no consideration has been given to the possibility that pre-existing urinary tract pathology or more advanced age of the animal might increase the susceptibility to renal and bladder injury. To these extents, therefore, the data cannot be related to the possibility of injury to persons in the older age groups or to individuals with renal disease.

The mild liver changes seen in rats and hamsters cannot be definitely assessed as to their importance in the evaluation of the possible hazards of polyoxyethylene stearates in the diet. There may be a possibility that pre-existing, subclinical injury to the liver might influence its susceptibility to further damage. In this same regard, the squamous cell hyperplasia in the stomach of the rat may be noted. Though this latter finding, and also the hemosiderosis in the liver of the hamster, each were reported only in single investigations, these studies were more detailed than most of the others, in some of which such changes may have been present though not found.

The observation (31) that two percent of dietary Myrj 45 markedly inhibited growth and hastened the onset of peripheral neuritis in rats on a basal diet containing 80 percent of the thiamine requirement introduces the question of its effects on borderline vitamin deficiencies in the population of the country. Investigation of the mechanism of the destructive effect of Myrj 45 on thiamine in a ration is necessary in order to evaluate this observation. Information available at this time indicates that the accelerated thiamine deficiency encountered when feeding low thiamine diets is caused by a destruction of thiamine in the ration before feeding.

In the toxicologic studies reviewed, a two percent level of polyoxyethylene stearate in the diet was the lowest concentration at which some effects were observed in experimental animals. The ineffective level has not been established. If 0.1 percent of polyoxyethylene stearate were consumed in the diet, the factor of safety would be no greater than 20 to 1. This margin is considered an inadequate range of safety even for young and healthy human subjects.

The introduction into a foodstuff of a new additive which does not positively contribute to the nutritional quality of the food presents a situation demanding a particularly conservative judgment. The use

of such an additive in a variety of basic foods which are unavoidably consumed by all groups within the population, in both health and disease, requires that sufficient evidence be obtained to provide a positive demonstration of harmlessness.

Were the additive in question a therapeutic agent, the appraisal of toxicologic data might be essentially a decision between two threats to health, *viz.*, the hazard of the untreated disease and the risk involved in treatment. As the necessity or justification for use becomes less apparent, the margin of safety demanded must be increased proportionately until, at the point where there are no benefits to the consumer directly referable to the maintenance or restoration of health, the justifiable risk approaches zero. Then the exercise of conservative judgment requires the demonstration of safety beyond any reasonable doubt.

V. Conclusions

The additional evidence available on polyoxyethylene stearate since this Committee's statement on the *Use of Surface Active Agents in Food* dated November 9, 1951 has been reviewed by the Food Protection Committee in the light of its Statement of Basic Principles.

No new food additive should be introduced for human consumption or continued use in the diet as long as a reasonable doubt remains concerning its safety. The potential toxicologic hazard in the incorporation of polyoxyethylene stearates into foods in technologically desirable quantities may not be great. Nevertheless, the experimental studies of the biologic behavior of polyoxyethylene stearate indicate the possibility of unfavorable reactions. The possibility of these outweighs the arguments advanced to support its use.

In the light of the above considerations, this Committee can only conclude that the available data fail to demonstrate that polyoxyethylene stearates are safe for use in foods under all patterns of dietary consumption and for all segments of the population.

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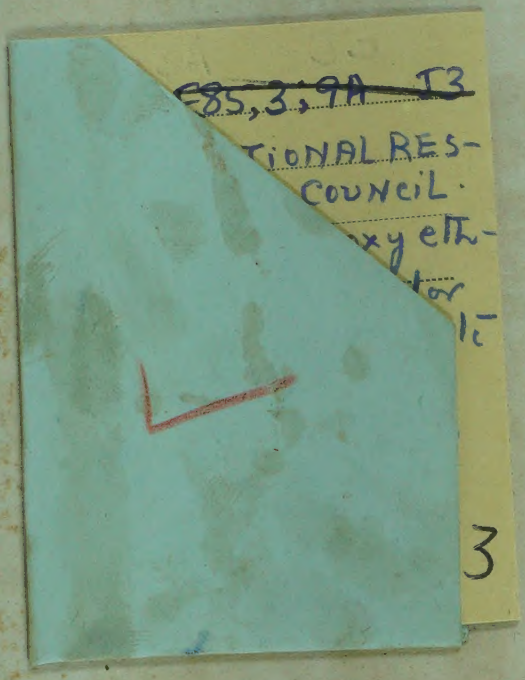
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